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Prevalence of serum Neutrophil Gelatinase Associated Lipocalin (NGAL) in Hepato-Renal Syndrome

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Abstract

Background: Hepatorenal syndrome (HRS) is a severe complication of cirrhosis which is characterized by renal dysfunction and associated with poor survival. Neutrophil gelatinase-associated lipocalin (NGAL) is a troponin-like biomarker for human acute kidney injury. **Objectives:** We aimed to measure the level of serum NGAL in patients with HRS and compare it to patients with compensated cirrhosis and with cirrhotic ascitic patients. **Methods:** A total of 45 patients with cirrhosis (15 compensated cirrhotic patients, 15 cirrhotic ascitic patients, and 15 cirrhotic patients with HRS) were included in the study. Blood samples were measured with Human NGAL sandwich ELISA. **Results:** Patients with HRS had significantly higher serum NGAL levels compared with patients with compensated cirrhosis and ascitic cirrhotic patients. Serum NGAL was significantly higher in patients with severe ascites as compared to those with mild or moderate ascites. Serum NGAL had a significant positive correlation with serum creatinine and BUN and a significant negative correlation with urinary Na and total serum proteins. **Conclusion:** We concluded that serum NGAL may be useful in cirrhotic patients, particularly in identifying HRS, but larger validation studies are still needed to confirm this observation.

Keywords: Hepatorenal syndrome; Neutrophil gelatinase-associated lipocalin; cirrhosis; ascites.

Introduction

Hepato-renal syndrome (HRS) is a distinct form of renal failure characterized by severe renal vasoconstriction that occurs in the setting of severe liver disease. HRS is the most frequent fatal complication of cirrhosis, because nearly half of patients die within 2 weeks of this diagnosis [1].

The annual incidence of HRS is estimated at 8% to 40% in cirrhosis. The Model for End Stage Liver Disease (MELD) score in patients with cirrhosis and ascites parallels the risk of developing HRS. Onset of ascites in patients with MELD scores of about 10 is associated with a 8% and 11% risk of HRS at 1 and 5 years, respectively. If the MELD score approaches 18 nearly 40% of patients develop HRS within 1year [2]. Clinical nephrology is discovering neutrophil gelatinase-associated lipocalin (NGAL), a small 25-kDa protein belonging to the lipocalin family, as one of the most promising biomarkers in the diagnostic field of acute kidney injury (AKI) [3].

Human Neutrophil Gelatinase Associated Lipocalin (NGAL), protein of the Lipocalin superfamily, was originally identified as a component, along with gelatinase (MMP-9), of a disulfide-linked heterodimer secreted by neutrophils, the protein is produced in

immature neutrophil precursors in the bone marrow and stored in specific granules for subsequent release. NGAL is also expressed at a low level in other human tissues, including the kidney, prostate, epithelia of the respiratory and alimentary tracts [4].

Materials and Methods

This study has been approved by the scientific and ethical Committee at Ain Shams University hospital, Cairo, Egypt. After written informed consent, 45 cirrhotic patients attending to Ain Shams University hospital were randomly enrolled. Patients were classified according to the presence of ascites and HRS criteria into three equal groups. Group I consists of 15 cirrhotic patients with normal renal function. Group II consists of 15 cirrhotic patients with ascites and normal creatinine. Group III consists of 15 cirrhotic patients with ascites and HRS. Exclusion criteria were; hepatocellular carcinoma, alcoholism, and chronic renal disease.

Diagnosis of HRS was established according to the International Ascites Club's diagnostic criteria of Hepatorenal syndrome (2007); patient suffering from cirrhosis with ascites; serum creatinine > 1.5 mg/dL; no improvement in serum creatinine after at least 2 days with diuretic withdrawal and intravenous volume expansion with albumin and absence of shock, nephrotoxic drugs administration, and parenchymal kidney disease.

Patients were subjected to full history taking and thorough clinical examination. Laboratory investigations include complete blood picture, ALT, AST, Bilirubin (total and direct), serum albumin, total proteins, PT, INR, creatinine, BUN, serum Na, serum K, urinary Na, fasting blood sugar, alpha feto-protein, and ascitic fluid chemical analysis.

For sNGAL, venous blood samples (1.0-1.5 mL) were taken. Blood samples were centrifuged at 2000 rpm for 10 minutes and the collected serum was stored at -80○C until assayed. Analysis of sNGAL was done using human-specific commercially available enzyme-linked immunoassays (ELISA) (R&D Systems, Inc., Minneapolis, Minnesota, USA) according to the manufacturer's recommendations. Concentrations of sNGAL were expressed in ng/mL.

Statistical methodology

Data was analyzed using SPSS version 17 (SPSS Inc., Chicago, IL, USA). Data was described as mean ± standard deviation for quantitative variables and. Student's t-test was used for paired and unpaired observations. A p value of < 0.05 was used to express significant statistical difference.

Results

A total of 45 cirrhotic patients were enrolled in the study. The mean age of the patients was 56.5 years ± 9.4 years, and 73.3% of the patients were males and 26.7% were females. Patients were divided into three equal groups according to the presence of ascites and HRS criteria. Demographic and biochemical characteristics of the study population are given in (Table 1). All groups were age and gender matched.

Table (1): Demographic and biochemical characteristics of patients:

	Cirrhosis (*n* = 15)	**Ascites (*n* = 15)**	**HRS (*n* = 15)**
Age (years)	42-60 (54.6±7.7)	49-70 (58.2±8.7)	40-80 (56.5±11.9)
Gender (female/male)	5/10	5/10	2/13
Creatine (mg/dL)	0.6-1.2 (0.9±0.19)	0.7-1.3 (0.96±0.25)	1.6-5.3 (2.83±0.97)
BUN (mg/dL)	8-18 (13.1±3.6)	11-19 (15.2±3)	19-65 (37.7±14.7)
ALT (U/L)	13-76 (44.5±22.9)	11-85 (42.2±23.1)	19-55 (35.1±11.5)
T. Bilirubin (mg/dL)	0.45-1 (0.73±0.19)	0.9-17.9 (4.9±4.9)	0.86-16.4 (4.9±3.9)
D. Bilirubin (mg/dL)	0.02-0.6 (0.21±0.17)	0.12-12.5 (2.8±3.4)	0.37-13.6 (3.6±3.4)
s. Albumin (g/dL)	3.4-4.4 (3.8±0.31)	1.7-3.3 (2.5±0.5)	1.9-3.3 (2.5±0.4)
U. Na (mEq/L/day)	95-200 (138±35)	50-192 (131±39)	20-150 (44±36)
s. Na (mEq/L)	135-144 (139±2.8)	120-144 (133±6)	117-145 (135±8)
s. K (mEq/L)	3.7-5.1 (4.4±0.5)	3.2-6.6 (4.4±0.9)	3.4-6.5 (4.6±0.9)
sNGAL (ng/mL)	0.5-5 (2.2±1.2)	2-6 (3.5±1.13)	3.5-8.5 (5.8±1.7)

Data analysis revealed no statistically significant intergroup differences as regarding the age, gender, serum K, and ALT. HRS group had a significantly higher creatinine, BUN, and significantly lower urinary Na as compared to cirrhotic and ascitic groups. Moreover, HRS group had a significantly higher direct bilirubin as compared to cirrhotic group. The HRS and ascitic groups had a significantly lower serum albumin and significantly higher total bilirubin than cirrhotic group. The ascitic group had a significantly lower serum Na as compared to cirrhotic group.

As regarding serum NGAL, the HRS group had a highly significant higher level as compared to both the ascitic group; p-value <0.001, and the compensated cirrhotic group; p-value <0.001. Moreover, the ascitic group has a significantly higher level as compared to cirrhotic group; p-value <0.05 (Figure 1).

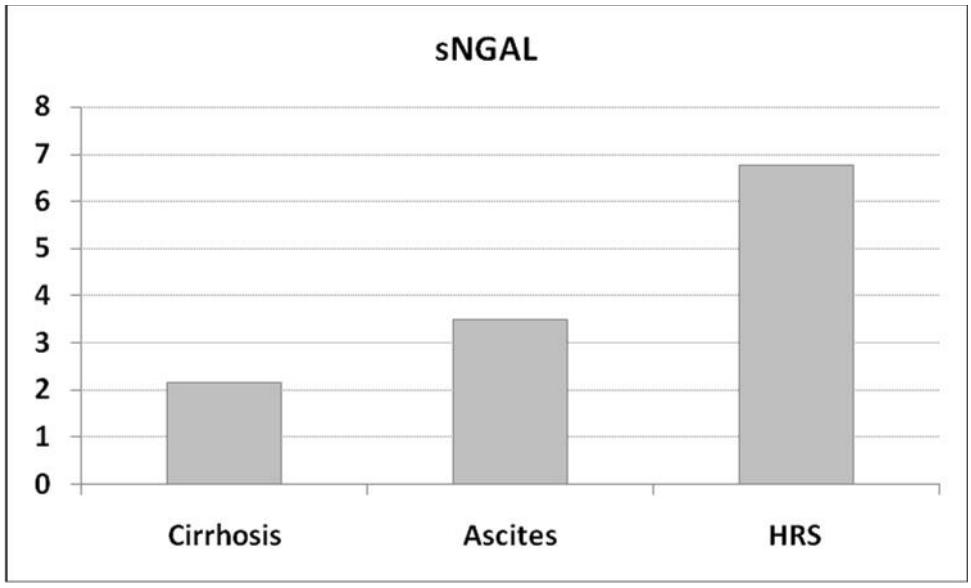

Figure (1): Serum NGAL in different groups.

Concerning the severity of ascites, serum NGAL was significantly higher in patients with severe ascites as compared to those with mild or moderate ascites. However, no significant difference was observed between mild and moderate ascites (Table 2; Figure 2).

Table (2): Comparative study between sNGAL and severity of ascites.

	Mild ascites	Moderate ascites	Severe ascites
sNGAL	2-7.5 (4.4±1.6)	2.5-5.5 (3.7±1.1)	3.5-8.5 (6.5±2)

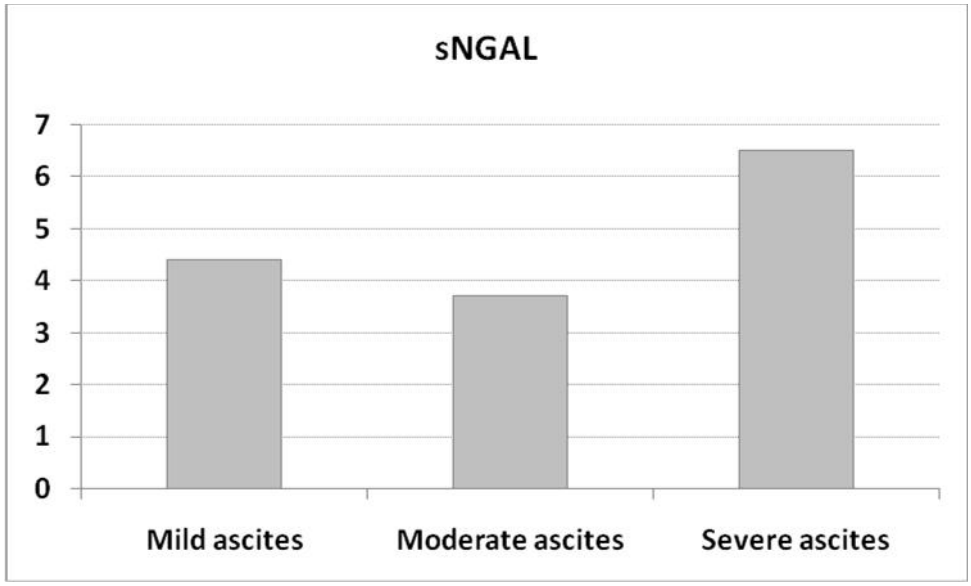

Figure (2): Serum NGAL according to severity of ascites.

Correlating serum NGAL to different parameters, sNGAL had a significant positive correlation with serum creatinine (r= 0.593, *p*= 0.001) and BUN (r=0.572, *p*=0.001) and a significant negative correlation with urinary Na (r=-0.572, p=0.001) and total serum proteins (r= - 0.771, p=0.001) (Table 3).

Table (3): Correlation coefficient between serum NGAL and different parameters.

	sNGAL			sNGAL	
	r	***p*-value**		**r**	***p*-value**
Age	-0.158	0.405	Serum Potassium (K)	0.190	0.314
ALT	-0.169	0.371	Ascitic Fluid leucocytes	0.009	0.973
Total bilirubin	0.104	0.586	Ascitic Fluid proteins	0.219	0.433
Direct bilirubin	0.177	0.359	Ascitic Fluid Glucose	0.044	0.876
Serum albumin	-0.047	0.803	Ascitic Fluid LDH	-0.286	0.301
Creatinine	0.593	0.001	Ascitic Fluid Albumin	0.088	0.755
BUN	0.572	0.001	Leucocytic count	0.221	0.429
Urinary Na	-0.620	0.000	Hemoglobin	0.293	0.289
P.T.	-0.167	0.377	Platelets	-0.056	0.844
I.N.R.	-0.051	0.790	Total proteins	-0.771	0.001
Serum sodium (Na)	0.268	0.153	Alpha feto-protein	0.076	0.787
Fasting blood sugar	-0.086	0.761			

Discussion

Chronic liver disease is common clinical problem in our country. Chronic liver disease involves a process of progressive destruction and regeneration of liver parenchyma leading to fibrosis and cirrhosis. Hepato-renal syndrome is a unique form of renal failure associated with advanced liver disease or cirrhosis and is characterized by functional renal impairment without significant changes in renal histology [5].

The evaluation of numerous exogenous and endogenous measures of kidney function continues to be the focus of much research in this patient population. NGAL is a 24 KDA glycoprotein that is expressed on the surface of neutrophils and epithelial cells, such as renal tubule cells, and may be over-expressed in ischemic and toxic kidney injuries. NGAL is present in plasma and urine; it can be detected in plasma and urine from AKI patients only 2 hours after kidney injury [6].

This study was undertaken to assess the validity of serum NGAL as a marker for hepato-renal syndrome in cirrhotic patients. Our results showed a highly significant higher sNGAL levels in cirrhotic patients suffering from HRS in correlation with compensated cirrhotic patients and even cirrhotic patients with merely ascites. Moreover, sNGAL level was significantly higher in ascitic cirrhotic patients as compared with compensated cirrhotic patients.

We found also that sNGAL was significantly higher in patients suffering from severe ascites as compared to those suffering from mild to moderate ascites. This could be explained by a probable kidney tubular injury in cirrhotic patients with severe ascites.

Our results are in agreement with Gungor and his colleagues [7], who reported high serum and urinary NGAL in patients with HRS in comparison to stable cirrhotic patients. Even, the same author concluded than sNGAL is higher in patients suffering from severe ascites as comparing to those with mild ascites. Also, Zhang and his colleagues [8] documented a high sNGAL in type 2 HRS.

The trend of the increase in sNGAL in our series agrees with the results obtained by many authors studied urinary NGAL [9, 10, 11]. They concluded that urinary NGAL was significantly higher in HRS as compared to compensated cirrhotics and ascitic patients.

The significant positive correlation observed between sNGAL with creatinine and BUN, was also observed by many authors [3, 7, 12].

On contrary, the significant negative correlation observed between sNGAL and urinary Na, demonstrates the increase in sNGAL with the progressive loss of renal ability to excrete Na. This observation was also documented by Shrestha and his colleagues [13].

The absence of significant correlation of sNGAL with liver function tests; namely ALT, bilirubin, INR, albumin, and PT, while the highly positive correlation with serum creatinine and BUN with the highly negative correlation with urinary Na and total proteins demonstrate the role of sNGAL in assessment of renal function in cirrhotic patients.

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